ORIGINAL ARTICLE

UDC: 611.3:611.899
DOI: 10.2298/VSP141231046M

Quantitative morphometric analysis of the myenteric nervous plexus ganglion structures along the human digestive tract

Kvantitativno-morfometrijska analiza ganglijskih struktura mijenteričkog nervnog spleta duž digestivnog trakta čoveka

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Abstract

Background/Aim. All the functions of the digestive system are controlled, guided and initiated by the autonomic nervous system. A special part of this system placed in the wall of the gastrointestinal tract is known as the enteric or metasympathetic nervous system. The aim of this study was to analyse myenteric nervous plexus in different parts of the digestive tract. **Methods.** We examined the myenteric nervous plexus of the esophagus, stomach, duodenum, jejunum, ileum, transverse colon and rectum in tissue samples taken from 30 cadavers of persons aged 20–84 years. After standard histological processing sections were stained with hematoxylin-eosin, cresyl violet (CV) and $AgNO_3$ method. Multipurpose test system M42 was used in morphometric analysis. The results were analyzed by *t*-test and analysis of variance. **Results.** The number of neurons *per* cm^2 surface was the lowest in the esophagus (2.045 ± 310.30) and the largest in the duodenum (65,511 ± 5,639). The statistical processing showed significant differences ($p < 0.001$) in the number of neurons between the esophagus and all other parts of the digestive tract. The maximal value of the average surface of the myenteric nervous plexus neurons was observed in the esophagus (588.93 ± 30.45 μm^2) and the lowest in the stomach (296.46 ± 22.53 μm^2). **Conclusion.** There are differences in the number of ganglion cells among different parts of the human digestive tract. The differences range from a few to several tens of thousands of neuron/cm^2. The myenteric nervous plexus of the esophagus was characterized by a significantly smaller number of neurons but their bodies and nuclei are significantly larger compared to other parts of the digestive tract.

Key words:
digestive system; myenteric plexus; neurons; cell count; histological techniques; cadaver.

Apstrakt

Uvod/Cilj. Sve funkcije digestivnog sistema su kontrolisane, usmeravane i inicirane od strane autonomnog nervnog sistema. Poseban deo ovog sistema, smešten u samom zidu delova digestivnog trakta, poznat je pod nazivom enterički ili metasimpatički nervni sistem. Cilj ovog istraživanja bio je da se prouči mijenterički nervni splet u različitim delovima digestivnog trakta. **Metode.** Ispitivan je mijenterički nervni splet jednjaka, želuca, duodenuma, jejunuma, ileuma, poprečnog kolona i rektuma u uzorcima tkiva uzetim od 30 kadavera, osoba starih od 20 do 84 godine. Nakon standardne histološke obrade preparati su bojeni hematoksilin-eozin, crezil violet (CV) i $AgNO_3$ metodom. Gotovi preparati su podvrgnuti morfometrijskoj analizi korišćenjem višenamenskog test sistema M42. Određivan je broj ganglijskih ćelija mijenteričkog nervnog spleta navedenih delova digestivnog trakta po jedinici površine (cm^2). Dobijeni rezultati obrađivani su *t*-testom i analizom varijanse. **Rezultati.** Broj neurona po cm^2 površine bio je najmanji u oblasti jednjaka (2 045 ± 310,30), a najveći u duodenumu (65 511 ± 5 639). Utvrđena je statistički značajna razlika ($p < 0,001$) u broju neurona između jednjaka i svih ostalih delova digestivnog trakta. Najveća vrednost prosečne površine neurona mijenteričkog nervnog spleta zabeležena je u jednjaku (588,93 ± 30,45 μm^2), a najmanja u želucu (296,46 ± 22,53 μm^2). **Zaključak.** Postoje razlike u broju ganglijskih ćelija između pojedinih delova digestivnog trakta čoveka. Razlike se kreću u rasponu od nekoliko, pa do više desetina hiljada neurona/cm^2. Mijenterički nervni splet jednjaka odlikuje se znatno manjim brojem neurona, ali su njihova tela i jedra znatno krupnija u odnosu na ostale delove digestivnog trakta.

Ključne reči:
digestivni sistem; plexus myentericus; neuroni; ćelije, broj; histološke tehnike; leš.

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Introduction

One of the basic features of the digestive tract is that it has its own nervous system, which extends from the pharynx to the rectum. All functions of the digestive tract are initiated and controlled by this system. The enteric nervous system contains a large number of nerve cells and nerve fibers, organized in the form of three large ganglionic (the myenteric – Auerbach's, the submucous – Meissner's, and the mucous) and more non-ganglionic nervous plexuses [1–3]. Enteric neurons form the ganglion plexus inside the wall of the gastrointestinal tract which are much more complex than any other component of the peripheral nervous system [4] and use a wide range of transmitters in very organized neural junctions [5]. Most regions of the gastrointestinal tract contain two main ganglion plexuses, the myenteric plexus between the longitudinal and circular smooth muscle layers, and the submucosal plexus, located in the connective tissue between the membrane and the circular muscle layer. *Plexus myentericus* is characterized by a dense network of nerve fibers that are interposed into many ganglions, usually grouped into nodes. Ganglions are diverse in terms of size, shape, number of neurons and structure. The shape, size and number of ganglia mainly depend on the characteristics intermuscular spaces that occupies. Many of myenteric ganglia are flat, irregular or lens- shaped and by observing to their longitudinal section surfaces they are usually outlined angular depending on the number and arrangement of the networks around them. In the myenteric nervous plexus are differentiated three interlaced network at the same plane (primary, secondary and tertiary plexus). Primary plexus represents quite a robust network of large nerve bundles that connect the ganglia by different sizes. This network of primary plexus shows only a longitudinal arrangement. The proximal border of distribution of myenteric plexus in humans is at 3–4 cm below the lower end of the larynx. Above this border, in the wall of the esophagus, can be seen only horizontally oriented, small bundles of nerve fibers without presence of ganglia, so is impossible to name these myenteric plexus. Some of studies [6, 7] on guinea pigs suggest that these nerve fibers participate in the innervation of striated muscle of the upper part of the esophagus. The highest density of ganglia and neurons in the human esophagus has been found at 4–6 cm above the posterior cardiac incisure [6]. In the distal direction the myenteric nervous plexus extends to the anus. Neurons of myenteric ganglia were larger in comparison to the other autonomic ganglions. Most of the enteric ganglion cells are multipolar, but unipolar, bipolar, ovoid, polygonal, or stellar neurons were also described. The number of neurons in the intestinal intramural ganglia is large, but varies from case to case [4], with different densities, different sizes of neurons in different segments of the alimentary canal. The smallest neurons are located in parts of the intestine closer to the attachement of the mesentery. In the myenteric system of guinea pig small intestine there are 14 types of neurons, each of which has a characteristic combination of morphological, neurochemical and biophysical features [8].

The main aim of our research was to establish neuronal density *per* cm² surface of the myenteric plexus of all main parts of the digestive tract, as well as, some of the basic morphological characteristics of myenteric neurons. Surely, also important is comparative analysis of the myenteric plexus different parts of the digestive system. Our research shows a very high neuronal density in all the parts of the alimentary tract.

We also tried to determine possible quantitative differences in the structure of the myenteric nervous plexus among these parts of the alimentary tract.

Methods

Tissue samples of the esophagus, stomach, duodenum, jejunum, ileum, transverse colon and rectum, with the consent of the Ethics Committee, were taken from autopsy material of the Institute of Forensic Medicine from 30 cadavers of both sexes, age range from 20 to 84 years. Samples were always taken from the same topographic places of the mentioned parts of the digestive tract (the middle part of the esophagus, central part of the anterior gastric wall, the middle of the upper part of the duodenum, anterior wall of the jejunum at 80 cm from duodenojejunal flexure, the anterior wall of the ileum 80 cm from the ileocecal junction, middle part of the free taenia of transverse colon and middle of the anterior wall ampullar part of the rectum). Thereafter, parts of samples (1 × 1 cm) were fixed in 10% buffered paraformaldehyde for 48 hours. After the routine processing through the series of alcohol, samples were embedded in paraffin blocks, and then cut into 6 μm thick section by two ways: sections perpendicular to the long axis of the anterior wall of the alimentary tract (classic), and longitudinal sections parallel to its long axis, serially from the serosa to the myenteric plexus, and through it. Sections were stained with hematoxylin-eosin method, but for identification of the ganglion structure and cells neighboring sections were stained with silver-nitrate (Mason-Fontana) and with cresyl-violet.

Silver-nitrate staining by the method of Mason Fontana included: hydrated sections were dipped in a previously prepared solution of silver-nitrate for 2 hours at 56°C, rinsed with distilled water and toning with a 0.2% solution of gold-chloride for 2–3 minutes, again rinsed with distilled water and 1 minute put down to the 5% sodium thiosulphate solution; again, rinsed with distilled water and dipped for 5 minutes in the nuclear-fast red color, then mounted on glass slides and cover with covering glasses. The result of staining was: argentaphilic granules in nerve cells were black, nuclei are pink-reddish and cytoplasm pale-pink.

Cresyl-violet staining for nerve cells included: hydrated sections were left in a previously prepared 0.5% solution of cresyl-violet for 30 minutes. After that, they were discolored in 96% ethyl-alcohol to which 1 drop of HCl was added, and discoloration controlled under a microscope. When the desired staining of sections was achieved they were dehydrated and mounted on glass slides. The result of staining were: dark blue nucleus, cytoplasm slightly lighter, while nerve fibers were not colored.

Quantification

Analysis was performed by the test system M42 calibrated to the proper magnification of light microscope (Carl Zeiss Jena). For measurements of the diameters of the cells and their nuclei we used an ocular micrometer calibrated to the appropriate magnification. At each section we analyzed 10 visual fields, and the obtained data were entered into spread sheets. The number of points of the test-system which fall on areas of ganglion structure was counted, as well as the number of points that fall on the profile of the myenteric plexus ganglion cells body. Also, the total number of neurons located within the area of ganglion structure was counted. Then we calculated the number of neurons *per* cm^2 surface of the nervous plexus, as well as the surface of individual profiles of the nerve cells bodies and their nuclei. Within morphometric analysis the surface of individual ganglion cells and the surface of their nuclei in different parts of the human digestive tract (relationship of the total surface profile of neurons to the surface area of the ganglionic structures in which they are located) were determined.

The results presented in the text and tables are expressed as mean ± standard deviation. The statistical significance (at the level of $p < 0.05$ or $p < 0.001$) between the mean values was estimated using Students *t*-test for independent samples and the analysis of variance (Med Calc statistical software 12.5.0.0.).

Results

The myenteric nervous plexus (ganglions interconnected by bundles of nerve fibers) forms a polygonal network interposed between the longitudinal and circular smooth muscle layers of the digestive wall. On cross sections the ganglionic structures of the myenteric plexus are of relatively small dimensions and within each of them we noticed a lower or higher number of interconnected neurons (Figure 1), some of them also connected to the muscle cells by nerve fibers. However, longitudinal sections through the longitudinal axis of the plexus allowed ganglion structures of different shapes and sizes to be clearly visible (Figure 2). Their obtained shape and size depended on the extent of specific section

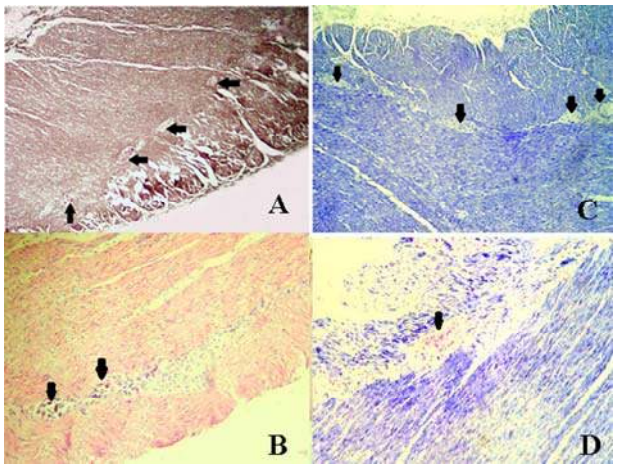

Fig. 1 – (A) Cross-section of the rectum wall, the arrow points to the myenteric ganglion (AgNO3, × 100); (B) Cross-section of the rectum wall, the arrow points to the myenteric ganglion [hematoxilin-eosin (HE), × 100)]; (C) Cross-section of the rectum wall, the arrow points to the myenteric ganglion [cresyl violet (CV), × 100)]; (D) Cross-section of the transverse colon wall, the arrow points to the myenteric ganglion (CV, × 200).

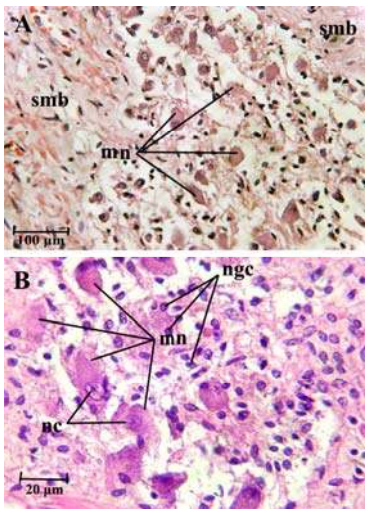

Fig. 2 – (A) Longitudinal section of the duodenum wall [hematoxilin-eosin (HE, × 400)]; (B) Longitudinal section of the stomach wall (HE, ×1000).
smb – smooth muscle bundles; mn – myenteric neurons; nc – nuclei of neurons; ngc – nuclei of glial cells.

affecting the plexus. Identification of neuronal bodies was clear on cresyl-violet stained sections (Figure 3). The result of this method of staining was neurons bodies coloring with unstained surrounding structures. Within the ganglion structures, which were generally arranged in groups of various size, the neurons of different shapes with vesicular nuclei which contain a very small quantity of chromatin were located. Around the neurons were located irregularly scattered oval nuclei of supporting or glial cells whose cytoplasm was not stained.

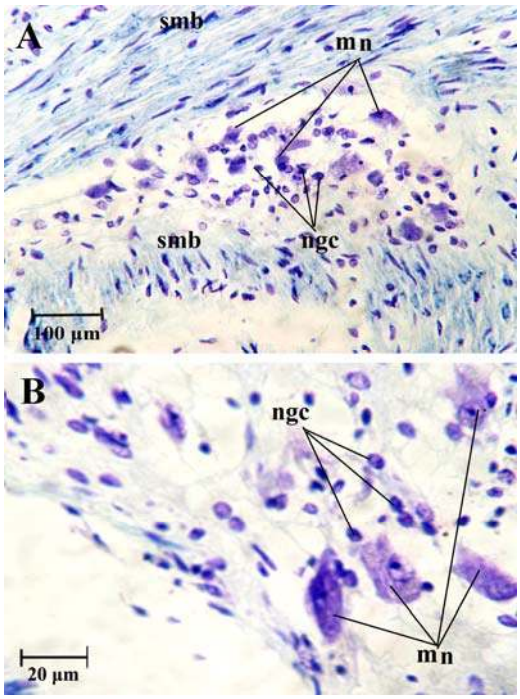

Fig. 3 – (A) Longitudinal section of the rectum wall [cresyl violet (CV), × 400]; (B) Longitudinal section of the stomach wall (CV, ×1000).
smb – smooth muscle bundles; mn – myenteric neurons; nc – nuclei of neurons; ngc – nuclei of glial cells.

Table 1 shows our results for the average number of myenteric plexus neurons *per* unit surface (cm^2). The lowest values were found in the esophagus (2,045 ± 310.30) and the largest ones in the duodenum (65,511 ± 5,639). There were noticeable very large differences in the number of neurons between the esophagus and of the other parts of the digestive tract. The results of measurements of the surface area (mm^2) of the myenteric plexus ganglionic structures along the alimentary tract are also presented in Table 1. The values found for surface area of ganglionic structures of different parts of the human digestive tract were different. The largest surface area was found in the stomach (0.01787 ± 0.0030 mm^2) and the smallest one in the duodenum (0.01185 ± 0.0016 mm^2). With the exception of the esophagus, statistical analysis (ANOVA/analysis of variance) did not show statistically significant differences at the level of $p < 0.05$ among investigated ganglion structures. However, we did not obtain data about surface of the esophagus ganglion structures, because during the study a significantly smaller number of ganglion cells was observed in the esophageal myenteric plexus, scattered in the intramuscular layer of the *tunica muscularis*. In addition, esophageal myenteric plexus ganglions were present in large parts of the sections only as a single (isolated). They were closely related to the circular and longitudinal layers of smooth muscle and only in some areas were in the groups and with a very smaller number of neurons. Therefore there was no possibility to include esophageal ganglionic structures into analysis by the same methodology which was used for the other parts of the digestive tract.

For complete analysis we also determined the surface which occupied the profiles of neurons within the ganglion structure, i.e. that phase of the surface of myenteric plexus ganglion structures which belonged to the profiles of nerve cells. The obtained values, presented in Table 1 (mm²), showed that the total surface neurons of the myenteric plexus ganglionic structure among the different parts of the digestive tract differed significantly. The largest difference (with the above explained exclusion of the esophagus) was between the stomach and transverse colon ($p < 0.001$).

The results of calculation of total surface percentages of the ganglion structures occupied by neuronal profiles are shown in Table 1. These data inform us about the density of plexus neurons in different parts of the digestive tube. The highest density of neurons we found in the area of duodenum (22.02%) and lowest one in the ileum (15.26%).

The results of the study of individual ganglion surface cells and of their nuclei (µm²) are shown in Table 2. The lar-

Table 1

Morphological characteristics of the myenteric plexus in the human digestive tract

Parts of Alimentary tract	Number of ganglion (cells/cm^2)	Surface area of ganglion structure (mm^2)	Surface phase of ganglion belonging to the profiles of nerve cells (mm^2)	
	x̄ ± SD	x̄ ± SD	x̄ ± SD	%
Esophagus	2,045 ± 310.30*	0	0	0
Stomach	46,260 ± 3,829	0.01787 ± 0.0030	0.00301 ± 0.00060	16.80
Duodenum	65,511 ± 5,639	0.01185 ± 0.0016	0.00261 ± 0.00035	22.02
Jejunum	53,794 ± 4,659	0.01344 ± 0.0016	0.00229 ± 0.00042	17.03
Ileum	44,850 ± 4,006	0.01395 ± 0.0021	0.00213 ± 0.00039	15.26
Transverse colon	37,836 ± 4,126	0.01227 ± 0.0017	0.00196 ± 0.00041*	15.97
Rectum	50,106 ± 3,004	0.01560 ± 0.0021	0.00260 ± 0.00041	16.66

x̄ – mean value; SD – standard deviation; *$p < 0.001$ compared to other groups (*t*-test);
****$p < 0.05$ compared to other groups (*t*-test).**

Table 2
Morphological characteristics of the myenteric plexus neurons in the human digestive tract

Parts of alimentary tract	Surface of neurons (μm^2) $\bar{x} \pm SD$	Surface of nuclei (μm^2) $\bar{x} \pm SD$
Oesophagus	588.93 ± 30.45*	82.88 ± 4.64*
Stomach	296.46 ± 22.53	37.19 ± 3.96
Duodenum	320.23 ± 29.39	38.97 ± 3.55
Jejunum	309.24 ± 27.76	38.14 ± 1.55
Ileum	318.05 ± 22.05	39.16 ± 2.60
Transverse colon	315.10 ± 19.97	37.72 ± 1.86
Rectum	303.04 ± 27.42	38.17 ± 1.86

$\bar{x}$ – mean value; SD – standard deviation;
***$p < 0.001$ compared to other groups (t-test);**
****$p < 0.05$ compared to other groups (t-test).**

gest average surface of myenteric plexus neurons was found in the esophagus (588.93 ± 30.45 μm^2) and the lowest one in the stomach (296.46 ± 22.53 μm^2). The only statistically significant difference ($p < 0.001$) we found between the surfaces of the esophagus ganglion cells and those of the myenteric neurons in other parts of the digestive tract. A similar distribution we obtained for nuclei, so that the maximal average value of neuronal nuclei surface (82.88 ± 4.64 μm^2) was observed in the esophagus and the lowest one (37.19 ± 3.96 μm^2) in the myenteric plexus ganglion cells of the stomach. The only statistically significant difference ($p < 0.001$) we found between the surfaces of nuclei of the esophageal neurons and those of the myenteric neuronal nuclei of all the other investigated parts of the digestive tract.

Discussion

In the available literature we found only several research reports on human myenteric nervous plexus. Our study shows that the myenteric plexus, on the cross sections of the wall of the digestive tract appears as a thin, wrinkled, interrupted lamellar structure inserted between longitudinal and circular layers of smooth muscle. In these sections the plexus is a relatively thin with uneven diameter, with thinner and thicker parts. Bodies of the nerve cells are grouped on the places of thickening.

For an appropriate and completed morphometric research and evaluation of the myenteric nervous plexus the longitudinal sections have proven useful. However, this requires a greater effort, with unavoidable continuous control of native section by light microscopy, which is necessary because a large number of serial sections going from serosa to mucosa. On the sections, along its longitudinal axis, the myenteric plexus appears in the form of smaller or larger groups of ganglion cells around which are scattered the bundles of longitudinal and circular smooth muscle.

The total number of neuron/cm^2 in the middle part of the esophagus was on the average 2,045 ± 310.30 indicating that the myenteric nervous plexus of the esophagus has much lower neuronal density/cm^2 than the other parts of the human digestive tract. Similar results for the lowest neurons in the esophagus reported Maifrino et al. [9] in the study on myenteric plexus of the rodent digestive tract. De Souza et al. [10] in quantitative research of the myenteric nervous plexus in the distal part of the human esophagus (11 cm from cardial orifice) found the highest density of ganglion cells in the segment of the esophagus, at 4 to 6 cm above the cardiac incisure. They reported variability of the neuronal density in human esophagus between 659 and 3,316 neuron/cm^2 that is similar to our results. Our analysis included the middle third or more proximal part of the esophagus. The neurons in the esophagus are quite dispersed, especially in the upper part, so the borders of ganglia should not be defined clearly. Generally, we found that moving across from cervical to thoracic segment of the esophagus the density and the size of myenteric ganglia increased.

In the available literature we did not find data about the number of neurons in the myenteric nervous plexus ganglion structures of the human stomach. Our results show the presence of enormously larger number of neurons in the myenteric plexus of the stomach as compared to the esophagus (average 46,260 ± 3,829 neurons/cm^2).

In our research the myenteric plexus of the duodenum showed a very high neuronal density (average 65,511 ± 5,639 neurons/cm^2). In the available literature we found no data for humans, but high neuronal density (52,000 – 55,300 neurons/cm^2) [11] was reported for the myenteric plexus of horse duodenum. In the duodenum of rats, 13,047 to 89,335 neurons/cm^2 depending on the age of animals were found from the oldest to the youngest animals, respectively [12].

It is important that we found a decreasing trend in the number of myenteric nervous plexus neurons in the parts of the small intestine going distally to the ileocaecal junction. The jejunum has a smaller number of neuron/cm^2 (53,794 ± 4,659) than the duodenum, and the ileum has smaller number of neuron/cm^2 (44,850 ± 4,006) in relation to the jejunum. In the absence of relevant data about the number of neurons in the human small intestine in the available literature we can only compare our results to those of Young et al. [13] of spatial density of myenteric neurons in the guinea pig ileum (17,300/cm^2), and of Miranda-Neto et al. [14] in the rat ileum in the area of 8.96 mm^2 where they found 1,647 ± 76.67 to 2,144.40 ± 161.03 neurons. In a rat, on the surface of 25.2 mm^2 Moreira et al. [15] found 6,648.6 ± 790.2 neurons. In guinea-pig small intestine the number of the myenteric plexus neurons follows decreasing sequence: duodenum > jejunum > ileum [16] what corresponds to our findings on humans.

In the available literature there was no study on the rating of the number of neurons in the human colon. In the myenteric plexus of the human transverse colon we found 37,836 ± 4,126 neurons/cm^2, but in rat colon de Araújo et al. [17] revealed the distribution 30,968 neuron/cm². High variations in the number of neuron/cm^2 depending of the colon part was shown to existe, even these differences were found in the single segment of the colon [18] depending on which region of the same level section was explored.

The rectum, similarly to the stomach shows a slightly higher average number of neuron/cm^2 (50,106 ± 3,004 neurons/cm^2) in relation to the ileum and transverse colon. This is probably because both these organs, unlike of other parts of the digestive tract, are characterized by the presence of very strong smooth muscle able for large amplitudes of alternating relaxation and a very strong propulsion.

Except the number of ganglion cell/cm^2 of myenteric plexus, our research included the areal surface of nerve structures in the parts of the digestive tract. Table 1 shows that the areal surfaces of the ganglion structures of the myenteric plexus ranged from 0.01185 ± 0.0016 mm^2 in the duodenum to 0.01787 ± 0.0030 mm^2 in the stomach. Neuronal section surfaces have a certain percent of ganglion structure surfaces (Table 1) and, the highest percent (22.02%) of the ganglion surface has the neurons of the duodenal myenteric plexus, corresponding to their large number.

There are no data showing that the phase surface belongs to myenteric neurons, as well as their possible differences within the digestive tract. In the effort to find out more about the morphological characteristics of neurons we focused our research on determining the nerve cells body surface and their nuclei (Table 2). The largest areas of neurons were observed in the esophagus (588.93 ± 30.45 μm^2). Liberti et al. [19] also found the highest value of pericaryonic fields (profiles of neurons) in the esophagus (489.97 ± 212.35 μm^2). Neuron body surfaces in other parts of the digestive tract in our research range from 296.46 ± 22.53 μm^2 (stomach) to 320.23 ± 29.39 μm^2 (duodenum), while similar to our results, Liberti et al. [19] have found smallest neuronal surfaces in the sigmoid colon (241.64 ± 122.62 μm^2), without significant differences between the mean values in the stomach (284.77 ± 134.70 μm^2) and duodenum (291.39 ± 157.86 μm^2). Smaller average size of neurons in the rat duodenum was found (229.7 ± 3.75 μm^2) [20] than in our study, maybe due to the variety of tissue processing methodologies and the ways of treatment and staining, as well as because of different sampling sites from the digestive tract. Future studies should use the most objective method of analysis for the enteric nervous plexus, as well as the standardization of staining for all nervous structures.

Conclusion

Longitudinal sections of the myenteric nervous plexus are necessary for its morphometric studies, to make them completely visible as a wide branching structure containing ganglion cells, surrounding glial cells and nerve fibers, clearly delineated from the surrounding smooth muscle bundles. Ganglion cells are large, oval, round or polygonal, eosinophilic cytoplasm with round eccentric vesicular nucleus and clearly visible nucleolus. Generally they are found in smaller or larger groups, and in rare cases (typically in the esophagus) as single neurons. The esophageal myenteric nervous plexus shows a less dense structure than the other parts of the digestive tract and is characterized by much larger neuronal surfaces and neuron nuclei compared to other parts of the digestive tract.

There are differences in the number of ganglion cells between the different parts of human digestive tract, ranging from a few to several tens of thousands of neuron/cm^2. Our results strongly indicate a specific decreasing gradation in density and size neurons within human digestive tract: duodenum > jejunum > rectum > stomach > ileum > colon transverse > esophagus.

Our results show specific morphometric characteristics of the myenteric plexus for each of the investigated parts of the human tract.

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Received on December 31, 2014.
Revised on May 7, 2015.
Accepted on May 12, 2015.
Online First March, 2016.